MOULTING, GROWTH, AND SURVIVAL AFTER EYESTALK REMOVAL IN UCA PUGILATOR

R. K. ABRAMOWITZ AND A. A. ABRAMOWITZ

(*From the Marine Biological Laboratory, Woods Hole, Mass.*)

Following the discovery of a chromatophorotropic hormone by Perkins (1928) and more especially, following the discovery of the sinus gland and other endocrine glands in the crustacean eyestalk by Hanström (1933–1937) and others, considerable interest has been shown in the extra-chromatophorotropic functions of the eyestalk. Hanström (1937) has pointed out that the endocrine glands discovered by him are present in various crustaceans which have either no chromatophores or show no metachrosis. Nevertheless, extracts prepared from the eyestalks of such animals are effective on the chromatophores of standard test animals. On these grounds, Hanström has suggested that the chromatophore hormone(s) may have other and more significant functions. It is also equally possible, however, that the eyestalk produces other hormones (besides the chromatophore activating system) which have significant functions in the life of the crustacean.

Among the various effects attributed to the eyestalks as based on experiments of either eyestalk extirpation or of the injection of eyestalk extracts, the relation between the eyestalks and the moulting process of crustaceans is of great interest. In our study of the specificity of the chromatophorotropic hormone of *Uca* (Abramowitz and Abramowitz, 1938), we observed that animals deprived of their eyestalks moulted more frequently than normal animals. We further remarked that the eyestalks did not seem to be essential to the life of this crustacean because we had maintained some blinded animals throughout the summer months (11 weeks in all) at the end of which time the experiments were discontinued. We have returned to these observations in order to obtain some quantitative data concerning these relationships. We were also stimulated to study in detail the effect of eyestalk removal on the viability of *Uca* because of the results obtained somewhat later by Brown (1938) on the crayfish, *Cambarus,* whose eyestalks are essential to its continued life.

The chronological development of this subject is quite interesting because the effects of eyestalk removal on moulting were known long before they were fully appreciated. Perhaps the first observation in this connection was that of Megašur (1912) who noted that the first and subsequent moults occurred earlier in blinded *Astacus vulgaris* than in normal animals. Koller (1930) found that less calcium was deposited in the exoskeletons of blinded animals than in those of normal animals. We (1938, 1939) also noted the moult-inhibiting function of the eyestalks of *Uca pugilator,* which moults more frequently following loss of its eyestalks. Hanström (1939) observed that the first moult took place earlier in blinded *Eriorcheir sinensis* than in normal specimens. Brown and Cunningham (1939) have established this relationship on a glandular basis for they were able to lower the high percentage of moulting in eyestalkless *Cambarus* by implanting the sinus gland. The literature on the viability effect is less uniform. Various investigators working with decapod crustaceans whose eyestalks had been removed reported survivals ranging from a few months to years (Megašur, 1912; Herbst, 1901; Abramowitz and Abramowitz, 1938). On the other hand, *Cambarus* succumbs within a few days following eyestalk amputation (Brown, 1938).

Materials and Methods

The experimental material consisted of two series of animals, one comprising slightly over a hundred specimens, and the other of 753 specimens. In the first experimental series, one hundred individuals (*Uca pugilator*) consisting of a mixed population of both sexes and of various sizes were blinded by excision of their eyestalks and placed in large crystallizing dishes for 4 or 5 days. After the fourth day, 76 animals, chosen at random, were segregated into 76 paper cups. Each animal was segregated because *Uca* is cannibalistic even if well fed, and is especially cannibalistic if allowed access to moulting individuals. Twenty-five unoperated animals were also segregated and maintained as controls. Both sets of animals were fed daily with small pieces of clam. Sea water, which was placed in the cups to a depth of about one-quarter of an inch, was changed daily. The experiment was continued for 48 days during which time observations on moulting, growth, and viability were made.

The second series (753 blinded animals) was used to check the rate of moulting found in the first series. The animals were maintained in large crystallizing dishes, about 30 specimens per dish. These animals were fed and given fresh sea water daily. No observations on viability were made in this series because many of the animals were devoured during ecdysis. This series was carried for only 30 days.

Results

Operative Mortality

The operative mortality was quite low in spite of the fact that the eyestalks were merely cut off with a pair of scissors without further measures. The usual operative mortality is 8 per cent, although it may rise to 12 per cent. All deaths occurring during the first 4 days following the operation were listed as operative deaths. In the large series of 753 animals, 8.7 per cent died within the first three days. After the first few days, the animals recover from the shock of the operation, and appear to be in good condition. They are vigorous, eat well, and show, at least superficially, no signs of serious weakness. Such a condition is maintained until the first moult, after which serious debilities appear. Some of these post-operative animals die at various times before their first moult, but these cases will be analyzed in another section. No observations were made with regard to a diurnal cycle of motor activity.

Moulting

In the first series of 76 animals, the first case of moulting occurred on the eleventh day following excision of the eystalks. By the sixteenth day, 50 per cent of the animals had moulted, and by the thirty-fifth day, all of the operated animals had moulted. The control group of animals showed quite different results. Only 7 of these animals had moulted during the 48 days of observation, the first case occurring on the twenty-fifth day after the experiment was started. The results are expressed in the form of curves (Fig. 1), showing the percentage of animals moulted at daily intervals following the operation. Such curves bring out more readily the accelerating effect of eyestalk removal on moulting.

The larger series of 753 animals showed a moulting rate essentially similar to that already described. The shape of the curve was approximately the same as that in Fig. 1, but was shifted farther along on the abscissa. The first moult occurred on the ninth day but the rate did not rise until the fifteenth day when 10 per cent of the animals had moulted. From this point on, the curve rose parallel to that in Fig. 1 but separated from it by a distance equal to 4 days on the abscissa. Thus, 25 per cent of the isolated crabs moulted on the thirteenth day whereas 25 per cent of the animals in the second series moulted on the seventeenth day. Fifty per cent of the isolated animals moulted on the sixteenth day whereas the same percentage of the non-segregated crabs moulted on the twentieth day. This difference became more noticeable, for only 80 per cent of the animals had moulted on the twenty-ninth day, as compared with the twenty-first day for the segregated animals. We are not in a position to state the cause of this difference in the time relations of moulting in segregated and non-segregated animals, but we wish to point out that a sufficiently large group of animals has been used to demonstrate the accelerating effect of eyestalk removal on ecdysis.

The percentages of moults (Fig. 1) were calculated on the basis of 70 animals, since 6 of the animals died before they had moulted. Only the first moults were included in this curve. Three of the animals moulted twice during the experiment, the second moult occurring between the twenty-first and twenty-sixth day after the first moult. Only 29 per cent of the control animals moulted during the 48 days, and none of these animals moulted a second time. Excluding the 3 cases in which

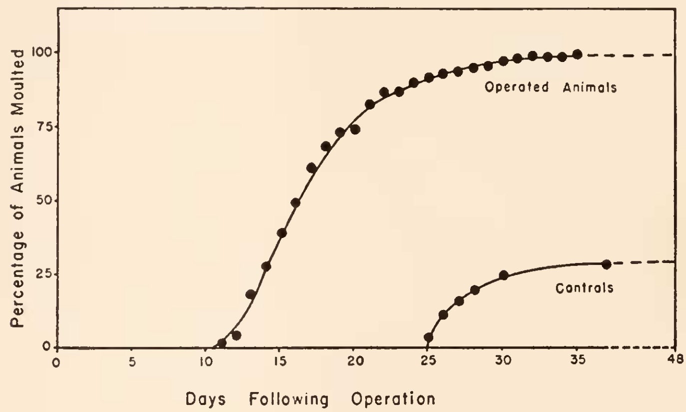

Fig. 1

moulting occurred twice, the degree of ecdysis in the operated group is about 300 per cent that of the control group over the same period of time. Furthermore, the onset of moulting in a mixed population chosen at random is two weeks earlier in the operated group than in the unoperated group. Finally, the loss of the eyestalks may shorten the intermoult period. The inter-moult interval, of course, was not determined since none of the normal specimens moulted twice during the period of observation. However, a shorter inter-moult interval may result from eyestalk removal since 3 of the operated animals moulted twice (between 21–26 days following their first moult) during the 48 days. The unoperated moulted animals were carried for only 20–23 days after their first moult and consequently it is not possible to decide whether these animals might have moulted within a short time after the experiment was discontinued. We feel, however, that the normal inter-moult period must be longer than 3–4 weeks since in a mixed population most of the animals would have moulted within this time. Yet only 29 per cent of the animals had moulted in 7 weeks.

Viability

The relation between viability and moulting is quite complex, as an analysis of our data will indicate. Brown (1938) has presented evidence for the existence of a chemical substance that is essential to the life of the crayfish, and Brown and Cunningham (1939) further suggest that the viability function of the eyestalks is different from the moult-inhibiting function. The basis of the latter is the fact that blinded

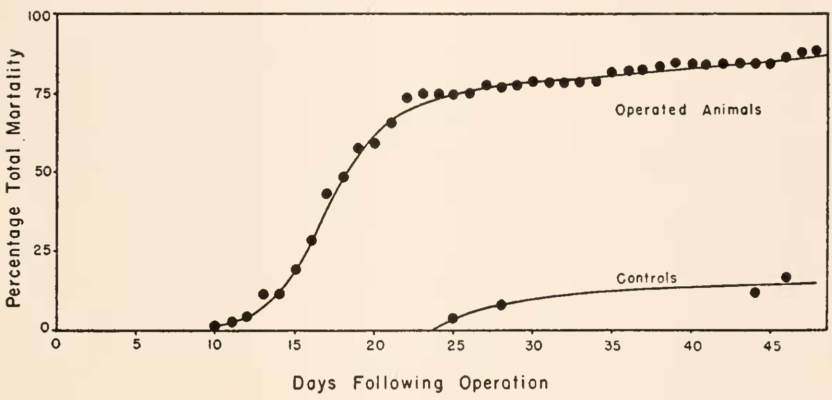

FIG. 2

animals sometimes continue to live several days after moulting and then die.

The total mortality in the control and operated groups of the first series of animals is shown in Fig. 2. The mortality curve is strikingly similar to that of the moulting curve, and gives the superficial impression that death and moulting bear a causal relationship. However, the analysis of the individual case histories reveals a more complex situation, and brings out more significant aspects of this relationship than is shown by a total mortality curve.

In the first series of animals, 7.9 per cent of the operated animals died directly without having moulted. In the second, larger series of animals, only 3.2 per cent succumbed before moulting. These deaths occurred from the tenth to thirtieth post-operative day. We regard these deaths as due directly to the loss of the eyestalks, since no complicating processes had intervened between operation and death. This mortality percentage is much too low to establish the presence of a life-sustaining secretion by the eyestalks, especially since 8.5 per cent of the unoperated specimens died in the same fashion. We conclude, therefore, that the loss of the eyestalks *per se* is not a cause of death in the species. As pointed out previously, many of the operated animals are in good shape for as much as 30–35 days but succumb shortly after moulting. If we exclude the 7.9 per cent of the animals dying before moulting, we can then state that mortality is coincident with moulting.

The total mortality over 48 days was 89 per cent in the operated group and 16.6 per cent in the control group. There can be no question, therefore, that the eyestalks are concerned in some way with viability. Since 8 per cent of the operated animals died before ecdysis, the total mortality during or some time after moulting is 81 per cent. Such a classification may not be justifiable for we cannot assume what the mortality might be provided the operated animals did not moult. Nevertheless, this division reveals a significant fact—that 91 per cent of all deaths occur during or shortly after moulting, and that only 9 per cent of the total deaths occur before moulting even though this latter takes place over a month. Correlated with these figures is the observation that the animals show no signs of impending death until after they have moulted in spite of the fact that some animals do not moult until a month or so after the operation. In the control group, half of the total deaths occurred before moulting, and half afterwards.

Nevertheless, 10.5 per cent of the total operated animals survived throughout the entire experiment. This constitutes 11.4 per cent of those animals which had moulted either once or twice, or 10.4 per cent of those animals which had moulted only once. On this basis we can also state that the eyestalks of this species have a function in addition to moult control as Brown and Cunningham (1939) have found for *Cambarus*. Thus, no definite answer can be given to the question of the separate control of moulting and viability in *Uca,* and the best we can do for the present is to give an estimate by a further analysis of our individual case histories of the relative proportions of a possible direct or indirect control of viability by the eyestalks.

The following table shows the percentages of 67 animals that moulted once and the time in days after moulting at which they succumbed:

Percentage	Days after moult
70	during or within 1 day
4.5	3
3.0	4
3.0	5
1.5	8
1.5	14
1.5	20
1.5	23
1.5	27
1.5	31

Of the animals which moulted twice, two died during ecdysis and one continued to live. Thus, about 75 per cent of the moulting deaths may be described as being due directly or indirectly to the process of ecdysis, while 25 per cent (which includes the viable 10.5 per cent) of the animals do not fall within this category.

Finally, another way of analyzing the data may be helpful. Part of the total moulting mortality, we feel, is due to mechanical difficulties in ecdysis, and part, probably a major portion, is due to some debility whose appearance is concomitant with or aggravated by the moulting process. We repeatedly observed that the animals have difficulty in moulting under the described environmental conditions. They appeared to have no difficulty in detaching themselves from the walking appendages and ventral abdominal segments, but the animals were frequently unable to remove themselves from the carapace and especially from the mouth parts. The scar tissue of the eyestubs also appeared to offer a mechanical difficulty. That two of the normal animals that moulted, or 28 per cent, also died during moult indicates the possible existence of such a mechanical factor which probably does not exist in the normal habitat. The remaining deaths are undoubtedly due to some severe deficiency. This must be true because 60 per cent of the animals which moulted once successfully, succumbed nevertheless in a weakened condition of a varying duration of time after moulting. Such a deficiency is not seen in the normal specimens, for those (72 per cent) which moulted successfully continued to live and were indistinguishable from normal specimens which had not moulted.

Growth

Since eyestalk removal accelerates moulting, it was expected that eyestalkless animals would attain a larger size. Due to the paucity of the animals which survived the 48-day experiment, we did not keep individual records of weights. However, the few animals which survived, especially one which had moulted twice, were virtually gigantic crabs.

In order to compare the experimental and normal specimens, we picked out the largest male and female specimens from a lot of 500 normal animals. These are shown in Plate I, together with the ordinary sized, and small specimens. The experimental specimens are also shown in Plate I. A glance at Plate I leaves little doubt that removal of the eyestalks leads to increased size.

Explanation of Plate I

The two columns at the left comprise 4 sets of normal animals chosen from a lot of 500 specimens, showing two intermediate stages from the largest male and female crabs to the smallest. The third column (at the right) shows 2 sets of animals whose eyestalks had been removed 48 days previously. All specimens are one-half natural size.

Miscellaneous Effects of Eyestalk Removal

Animals deprived of their eyestalks seem to lose pigmentation, an effect which becomes evident especially after moulting. Normal animals have a blackish carapace in which purple and red colors may also be seen. After eyestalk removal, the entire animal pales noticeably—a well-known effect due to the concentration of melanophore pigment. The carapace of blinded animals is much paler than that of a normal specimen, though not the cream yellow color of the walking legs. Although there is considerable variation, the shade assumed by the carapace may be described as blackish gray. After moulting, the carapace assumes a grayish-white appearance, which may be due to the loss of pigment. The moulted animal sometimes gives the appearance of an albino specimen. Either pigment has been lost, or sufficient replacement has not taken place after ecdysis. More cannot be said until further study.

Uca breeds during September, as indicated by the appearance of large masses of eggs, copulation, and finally shedding of the eggs. Animals without eyestalks have been observed to copulate and shed their eggs. However, such animals were blinded for only a few weeks before the onset of the breeding season and thus sufficient time may not have elapsed for any effect on reproduction to take place. This is worth investigation, however, for as yet no endocrine influence on the reproductive system of crustaceans has been demonstrated.

Summary

After removal of the eyestalks in *Uca pugilator,* all of the animals moulted within 35 days after the operation, and a few of the animals moulted twice within 48 days. Only 29 per cent of the normal animals moulted within 48 days, and none moulted twice. Eyestalk removal not only accelerated moulting but shortened the time in which the first and second moults occurred. The time at which the same percentage of moulting occurred was somewhat greater for non-segregated than for segregated animals.

Viability is related to moulting, and most of the deaths following eyestalk removal in this species are due in part to a mechanical difficulty and in part to some severe deficiency occurring during or aggravated by ecdysis. There is insufficient evidence to decide whether the eyestalks secrete a specific, life-sustaining hormone directly responsible for the continued life of the animal, or whether the viability effect is in some way dependent on the moult-inhibiting function of the eyestalk. We do not believe that the loss of the eyestalks *per se* is directly responsible for the total mortality seen in eyestalkless crabs because of the negligible percentage of deaths occurring from 1 to 5 weeks before moulting, and because operated animals live for 5 weeks without showing any external signs of serious debilities only to succumb after ecdysis. Seventy-five per cent of the total mortality can be related directly or indirectly to ecdysis, while 25 per cent (which includes the 10.5 per cent viable specimens) does not fall in this category and may be cited in support of a differentiation of the viability and moult-inhibiting functions of the eyestalk.

Eyestalk removal also leads to increased size. Some of the eyestalkless specimens 48 days after the operation have attained gigantic proportions. Pigmentation also seems to be lost, an effect which is noticeable after the first moult. No effect of eyestalk removal has been noted on copulation and shedding of eggs.

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